

Had a look Nearly there Nailed it!

Microscopy

Apart from a very few special cases, cells cannot be seen with the naked eye. Both light microscopes (LM) and electron microscopes (EM) are used to magnify them and their structures.

Size matters

Rarely, cells can be seen with the naked eye. The biggest bacterium known is about 0.3mm (300µm), visible, just by comparison:
 • red blood cell = 8µm
 • a mitochondrion = 4µm

So, these are far too small to see without a microscope.

Magnification
 Image size = actual size × magnification
 If an image of a temple is 3cm wide at a magnification of ×1000, the actual width of the specimen would be $3 \div \frac{1000}{1} = 0.003 \text{ cm}$.

Magnification and resolution

It seems obvious that we must magnify small cells and structures so that they can be seen, but that is only part of the solution.

The other important factor is **resolution** - being able to see two objects as distinct.

The human eye has a resolution of about 0.1mm; two objects closer together than this will look like one object.

An LM can resolve to about 0.2µm and the EM to about 1nm or less. This depends on the wavelength (λ), for light this is about 500nm, for an electron beam less than 1nm.

Does your eye resolve well? It is worth magnifying a lot. Without high resolution, high magnification just gives something big, but still blurry.

Measurements

Measurement (µm)	Resolution (nm)	Magnification (x)	Resolution (nm)	Magnification (x)	Resolution (nm)	Magnification (x)
1000	1000	1	1000	1	1000	1
100	100	10	100	10	100	10
10	10	100	10	100	10	100
1	1	1000	1	1000	1	1000

Worked example

The electron micrograph image shows a ribosome.



Calculate its actual length between W and Y in µm, to two significant figures. (2 marks)

The image length is 68nm and the magnification is 6000. So, actual size = length ÷ magnification 6000 = 11µm

Achieving greater magnification and resolution

Magnification magnify a magnified image again, e.g. 10 times × 10 times = 100x

Resolution use radiation (light or electrons) of a shorter wavelength.

Empty magnification

If we magnify something beyond the resolving power no more detail is seen, the magnification is said to be empty.

Now try this

Explain why an EM has a much better resolving power than an LM. (2 marks)

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Practical microscopy

In order to see as much detail as possible when looking at something through a microscope, preparation of the specimen is needed. Cutting of sections and staining are common techniques in light microscopy.

The light transmission microscope

Light microscopy works when light passes through a specimen so that it can then enter the eye. This means the specimen must be thin.

An onion leaf scale epidermis has a single layer of cells which can be peeled off and viewed easily. But most other structures need to be sectioned.



Light from the blue part of a red onion.

Staining

If a section is obtained and put straight under the microscope the results can be disappointing. Most structures are colourless in their natural state.

Staining is used to give structures colour and contrast and allow identification.



Plant leaf section showing lignin that stains a part of the stem vascular bundle.

Common stains used:

- iodine solution - stains starch blue/black
- ethanolic carmalum - stains DNA reddish-purple
- methylene blue - stains nuclei and chromosomes blue
- phosphotungstic - stains lignin, found in plant cells, red.

The electron microscope

The electron microscope (EM) has revolutionised our understanding of cells. It is not of great use, though.

- ✗ All specimens are examined in a vacuum so it is impossible to look at living material.
- ✗ The treatment is likely to result in artefacts that is things which are not really there but appear due to the preparation methods.
- ✗ EMs are still extremely expensive to buy, run and maintain.

Sectioning
 A sharp razor blade can be used, with practice, to cut the sections.

Using a blade to cut the sections

Worked example

Evaluate the strengths and weaknesses of EM and LM microscopes. (2 marks)

An EM has much better resolution and therefore magnification is not 'empty', up to very high values. However, specimens cannot be alive and there is so much harsh treatment in the preparation of materials it is not always clear what is real and what is artefact.

An LM has limited resolution so magnification above about 6000x is pointless. Many organisms are too small to see at this power. However, living material can be viewed and the problem of artefacts is much less severe.

Now try this

What eyes would you use to take the dimensions of onion root tip cells? (2 marks)

Guide To Microscopy

RS Peters



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